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Structural elements of primary CCR5-using HIV-1 gp120 proteins influencing sensitivity and resistance to the broadly neutralizing monoclonal antibody b12

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ABSTRACT

Structure-guided approaches to HIV-1 vaccine design depend on knowledge of the presentation of neutralizing epitopes on gp120, such as the epitope for the broadly neutralizing mAb b12. Here, we characterized predicted three-dimensional structures of functionally diverse gp120 proteins in their b12-bound conformation, to better understand the gp120 determinants that expose or occlude the b12 epitope. Mapping the gp120–b12 binding interface identified amino acid polymorphisms within the C2, C3, C4 and V5 regions of gp120 associated with augmented b12 binding, and importantly, identified residues in the b12-exclusive binding domain of gp120 that are important for b12 neutralization resistance. Structural studies suggest that these b12 resistance variants promote reduced conformational flexibility in the b12 recognition site, which we show involves structural alterations within the gp120 CD4 binding loop and the V4 loop. Together, our studies provide new mechanistic insights into the gp120 determinants influencing sensitivity and resistance to HIV-1 neutralization by b12.

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Introduction

The envelope glycoprotein complex (Env) of human immunodeficiency virus type 1 (HIV-1) exists as a trimer of heterodimers, comprising the surface gp120 glycoproteins non-covalently linked to gp41 transmembrane glycoproteins that embed the complex into the enveloping lipid bilayer (Chan et al., 1997; Kwong et al., 2000a, 1998). The gp120 glycoprotein is primarily responsible for interaction with host cell molecules used for viral attachment and entry. The first step in the HIV-1 entry process involves gp120 binding to the CD4 molecule, which is a high affinity interaction that facilitates viral attachment (Dalgleish et al., 1984). gp120 binds to CD4 via the Phe43 cavity, which is a conserved hydrophobic pocket created by residues located within the inner domain, bridging sheet and outer domain of gp120 (Kwong et al., 1998; Wyatt et al., 1998). CD4 binding draws the viral membrane into close proximity to the cellular membrane via bending of a flexible hinge region in CD4 (Sattentau, 1998). Conformational changes induced in gp120 as a result of CD4 binding lead to the exposure of a binding site for one of the chemokine coreceptors used for HIV-1 entry (Kwong et al., 1998; Rizzuto et al., 1998; Sattentau and Moore, 1991; Wu et al., 1996; Wyatt et al., 1995), which are either CCR5 or CXCR4 (Deng et al., 1996; Dragic et al., 1996; Feng et al., 1996).

The gp120 protein consists of highly conserved inner and outer domains, named for their relative position in the context of the Env spike, as well as the bridging sheet which is a distinct structure comprising elements from the inner and outer domains (Kwong et al., 1998). The outer domain is exposed on the surface of the Env trimer and has therefore evolved structural features that protect gp120 from the humoral and cellular immune responses (Kwong et al., 2002), including five highly variable loops (named V1 through V5) and a heavily glycosylated surface.

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In spite of these defence mechanisms, the outer domain also contains important immunological epitopes that are recognized by the few highly potent and broadly neutralizing antibodies discovered thus far (Wyatt et al., 1998; Wyatt and Sodroski, 1998), making the outer domain of particular interest for vaccine development (Yang et al., 2004). The inner domain faces the trimer axis and interacts with gp41 via the N- and C- termini (Kwong et al., 1998, 2000b; Wyatt et al., 1998), and contains receptor and coreceptor binding site structures that are highly conserved amongst HIV-1 strains. The V1/V2 loops emanate from distal strands of the inner domain and contribute to conformational masking of the CD4 and coreceptor binding sites (Kwong et al., 1998, 2000b; Wyatt et al., 1993). The anti-parallel bridging sheet forms a highly conserved structure that contains residues critical for coreceptor binding that are exposed after binding to CD4 (Kwong et al., 1998; Rizzuto et al., 1998; Thali et al., 1993; Trkola et al., 1996a; Wu et al., 1996). Extensive glycosylation and sequence variability within the loop regions enable HIV-1 to evade the host immune response, and are particularly important in protecting key target cell receptor binding sites from neutralization by antibodies (Kozarsky et al., 1989; Kwong et al., 2002; Wyatt et al., 1998).

The presentation of Env on the surface of the virion exposes gp120 to a myriad of defence mechanisms by both the cellular and humoral arms of the immune system. However, the majority of antibodies targeting gp120 are ineffective at neutralizing HIV-1 infection (Burton et al., 1994; Wyatt et al., 1998). Crystallographic analysis of gp120 in complex with the rare broadly neutralizing antibody b12 has provided valuable insights into the nature of a highly conserved neutralization epitope on gp120 (Zhou et al., 2007). The b12 monoclonal antibody (mAb) binds to an epitope that overlaps the CD4 binding site on gp120 (Burton et al., 1994; McInerney et al., 1997), and can neutralize primary HIV-1 strains from all HIV-1 subtypes (Burton et al., 1994; Trkola et al., 1995) as well as protect macaques from vaginal challenge with chimeric simian-HIV (SHIV) (Parren et al., 2001; Veazey et al., 2003). CD4 and b12 both bind predominantly to the same Env surface exposed on the outer domain of gp120. However, b12 binds to gp120 with greater affinity than CD4, presumably because b12 grasps a highly conserved structure referred to as the CD4 binding loop with all three loops of its heavy chain (Zhou et al., 2007). In comparison, CD4 only binds to one side of the CD4 binding loop. The angles of approach by CD4 and b12 are also slightly different, resulting in binding epitopes that are distinct but which have considerable overlap (Zhou et al., 2007). Moreover, cryo-electron tomography studies of b12-bound trimeric Env complexes showed that b12-induced conformational changes in gp120 were less extensive than those induced by CD4 (Liu et al., 2008). Another significant difference between the b12 and CD4 binding sites involves the β20/β21 strand of gp120. The β20/β21 strand is one of the initial contact sites for CD4 and becomes part of the bridging sheet after CD4-induced conformational changes bring the β2/β3 strand of the inner domain and β20/β21 strand into close proximity (Kwong et al., 1998). While previous mutagenesis studies suggest that there is no appreciable effect of β20/β21-strand mutations on gp120–b12 binding (Zwick et al., 2003), more recent studies suggest a significant effect of this gp120 region on b12 binding (Li et al., 2011). Together, these data suggest that while the CD4 binding site and b12 epitope may encompass some overlapping regions of gp120, they are also independently modulated by different structures within gp120.

Although the b12-bound crystal structure of the CXCR4-using HXB2 strain of gp120 has provided valuable insights into the presentation of an important neutralizing epitope on gp120 (Zhou et al., 2007), comparatively little is known about the structure and presentation of this epitope on more clinically relevant CCR5-using (R5) gp120 proteins. Therefore, in this study we used homology modeling to study the structural features of functionally diverse and physiologically relevant primary R5 gp120 proteins in their b12-bound state. These gp120 proteins have well characterized functional phenotypes including fusogenicity, entry kinetics, the efficiency and mechanism of CCR5 engagement, as well as exposure of the CD4 binding site (Sterjovski et al., 2007', 2011', 2010). By mapping the b12 structural epitope to the amino acid sequences of primary gp120 proteins with a range of b12-binding and b12-neutralization sensitivities, we identified several polymorphisms at the gp120–b12 interface associated with sensitivity and resistance to b12. By comparing the b12 epitope to the overlapping yet distinct CD4 binding site we were able to identify regions that were important for b12 resistance that mapped exclusively to the b12 epitope. Our structural studies also illustrate the importance of gp120 structural flexibility for efficient presentation of the b12 epitope. Together, our results provide new mechanistic insights into the interaction between physiologically-relevant gp120 proteins and b12, which may be significant for structure-guided approaches to HIV-1 vaccine design that depend on a thorough knowledge of the presentation of neutralization epitopes on gp120.

Materials and methods:

Cells

293 T cells were cultured in DMEM supplemented with 10% (vol/vol) fetal calf serum (FCS), and 100 μg of penicillin and streptomycin per ml. JC53 cells (Platt et al., 1998), were cultured in DMEM supplemented with 10% (vol/vol) FCS, and 100 μg of penicillin and streptomycin per ml.

Plasmids

The HIV-1 Envs used in this study were cloned from primary R5 HIV-1 isolates, which have been described in detail previously, including the clinical characteristics of the subjects from whom they were isolated (Sterjovski et al., 2007', 2011', 2010). The Envs used were NB2-C1, NB2-C4, NB6-C3, NB6-C4, NB7-C1, NB7-C2, NB8-C2, NB8-C4, NB23-C2, NB23-C3, NB24-C3, NB24-C4, NB25-C2, NB25-C3, NB27-C2, and NB27-C3, (Sterjovski et al., 2007', 2011', 2010) which are cloned into the pSVIII-Env expression vector (Gao et al., 1996). The pCMVΔP1ΔenvpA and pHIV-1Luc plasmids used to generate Env pseudotyped luciferase reporter viruses have been described previously (Yang et al., 2005', 2004', 2001).

Production and titration of env-pseudotyped luciferase reporter viruses

Env-pseudotyped, luciferase reporter viruses were produced by transfection of 293 T cells with pCMVΔP1ΔenvpA, pHIV-1Luc and pSVIII-Env plasmids using Lipofectamine 2000 (Invitrogen) at a ratio of 1:3:1, as described previously (Cashin et al., 2011; Gray et al., 2006; Sterjovski et al., 2007; Yang et al., 2001). Supernatants were harvested 48 h later, filtered through 0.45 μm filters and stored at −80 °C. The $TCID_{50}$ of virus stocks was determined by titration in JC53 cells (Platt et al., 1998), as described previously (Gray et al., 2009; Roche et al., 2011a, 2011b).

Antibodies

The monoclonal antibody IgG1–b12 (b12) (Burton et al., 1991', 1994) and the polyclonal antibody HIV-Ig have been described previously (Burton et al., 1991, 1994; Muster et al., 1994; Trkola et al., 1995, 1996b). The BB10 pooled HIV-1+ sera has been

described previously (Gorry et al., 1999). Goat anti-human Ig (H+L chain) FITC labeled F(ab')$_2$ fragment was obtained from Chemicon (Merck Millipore, Billerica, MA).

Binding assays

Measurement of b12 binding to Env was determined using a cell-based binding assay that we have described previously (Sterjovski et al., 2011, 2010). Briefly, 293 T cells were transfected with Env expression plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturers' protocol, and stained for surface Env expression using pooled polyclonal BB10 HIV-1+sera, as described previously (Sterjovski et al., 2007). In this system, Env typically exists as monomers of gp120 and uncleaved gp160 (Gorry et al., 2002). Approximately 2×10^5 293 T cells transfected with each Env expression plasmid were used in binding reactions. Following pre-incubation in FACS buffer [phosphate buffered saline (PBS) containing 10% (vol/vol) FCS and 0.05% (wt/vol) sodium azide] for 1 h on ice, 293 T cells were washed twice in 1 ml of ice cold FACS buffer and resuspended in 50 μl of FACS buffer containing 1 μg b12/ml, a concentration determined empirically to be within the linear range of binding (data not shown). Cells were incubated on ice for 1 h then washed twice with ice cold FACS buffer before being resuspended in 50 μl of FACS buffer containing goat anti-human Ig (H+L chain) FITC labelled F(ab')$_2$ fragment (1:200 dilution). Following incubation with the secondary detection antibody for 1 h on ice, cells were washed twice and resuspended in 150 μl of ice cold PBS containing 4% (wt/vol) paraformaldehyde, and analyzed by flow cytometry as described previously (Gorry et al., 1999).

Neutralization assays

The ability of b12 and HIV-Ig to neutralize the infectivity of Env-pseudotyped luciferase reporter viruses was assayed using JC53 cells. Two hundred $TCID_{50}$ of each Env-pseudotyped luciferase reporter virus, equating to a multiplicity of infection (MOI) of 0.02, was incubated with 10-fold increasing concentrations of b12 (0.0005 to 50 μg/ml) or HIV-Ig (1 to 10,000 μg/ml) for 1 h at 37 °C. The virus/antibody mixtures were then used to inoculate JC53 cells plated in a 96-well plate and incubated overnight at 37 °C. Cells were rinsed twice with culture medium to remove residual virus inoculum and incubated a further 48 h at 37 °C. Virus infectivity was then measured by assaying luciferase activity in cell lysates (Promega, Madison, WI) according to the manufacturer's protocol. Negative controls included mock-infected cells that were incubated with culture medium instead of virus, and cells treated with luciferase reporter virus pseudotyped with the non-functional ΔKS Env (Etemad-Moghadam et al., 2000). After subtracting background luciferase activity, the amount of luciferase activity in the presence of antibody was expressed as a percentage of the amount produced in control cultures containing no antibody. The percent inhibition was calculated by subtracting this number from 100. Data were fitted with a non-linear function, and fifty- and ninety- percent inhibitory concentrations (IC_{50} and IC_{90}, respectively) were calculated by least squares regression analysis of inhibition curves (Gorry et al., 2007, 2002; Gray et al., 2005).

Computer-aided structural modeling of gp120

Homology models of b12-bound primary gp120 sequences were prepared using the Build Model protocol of the Discovery Studio suite, version 1.6 (Accelrys). This approach uses the Modeler algorithm to generate an atomic model of the target protein from a template molecule and a sequence alignment. The template-based models are optimized by iterative cycles of conjugate-gradient minimization against a probability density function that includes spatial restraints derived from the template and residue specific properties (Sali and Blundell, 1993). The crystal structure of gp120 bound to b12 was used as a template for b12-bound models (PDB ID: 2NY7) (Zhou et al., 2007). Primary gp120 sequences were aligned against the 2NY7 sequence for the b12 models. The V1/V2 and variable loops were deleted and the V4 variable loop truncated from primary gp120 sequences. Chain breaks were inserted to mirror chain breaks in the crystal structure. N- and C- termini overhangs were cut using the modeling protocol. The coordinates for gp120 and the b12 heavy and light chains were extracted from the 2NY7 crystal structure. Sequence alignments were generated between 2NY7 gp120 and the primary gp120 isolates. The sequences for the b12 heavy and light chains were included as additional polypeptide chains so that the models of gp120 were constructed as complexes with the b12 heavy and light chains. To examine mutations in the context of a full-length V4 loop, additional models were generated for selected gp120 sequences where the V4 loop was left intact. Once the models were generated, harmonic constraints were applied prior to optimization using the Steepest Descent protocol, as we have described recently (Cashin et al., 2011).

Results and discussion

Homology models of primary R5 gp120 proteins bound to neutralizing antibody b12

To investigate structural alterations in gp120 associated with sensitivity to b12, predicted three-dimensional structures of 16 well-characterized primary gp120 proteins (Sterjovski et al., 2007, 2011, 2010) in their b12-bound state were generated using homology modeling. Fig. 1A shows the crystal structure of a stabilized variant of HXB2 gp120 (grey) in complex with the heavy and light chains (dark blue and light blue, respectively) of a b12 Fab fragment (PDB ID: 2NY7) that was used as a template to generate the b12-bound gp120 models. Primary gp120 model structures (grey) were superimposed onto the template structure (blue) (Fig. 1B) and structural similarity to the template crystal structure was determined by measuring the root mean square deviation (RMSD) (Table 1). RMSDs ranged from 0.22 Å to 0.44 Å, indicating a highly conserved core domain. Overall, there was little deviation between the b12-bound primary gp120 models and their crystal structure template, indicating a high degree of structural similarity between the crystal and model structures. It should be noted however, that since stabilization of gp120 in a CD4-bound state was required to solve the crystal structure of gp120 in complex with b12 (Zhou et al., 2007), these structures represent a gp120 form that exists closer to a CD4-bound state rather than to the native, unliganded gp120 structure.

Mapping the non-linear b12 epitope on primary gp120 structural models

We next mapped the three—dimensional b12 epitope on the amino acid sequence of the primary gp120 proteins. Residues located at the gp120–b12 interface were identified using the PISA computational platform (Krissinel and Henrick, 2007). Fig. 2 shows the molecular surface of the 2NY7 crystal structure (Fig. 2A) with gp120–b12 interface residues highlighted on both gp120 and b12 (Fig. 2B). Amino acid sequences at the gp120–b12 interface were extracted from the primary gp120 models and aligned against the gp120 protein sequence of the 2NY7 crystal structure (Fig. 2C–I). Interface residues were generally conserved across all gp120 sequences and overlapped with four regions of gp120 previously determined to be part of the CD4 binding domain (Kwong et al., 1998; McCaffrey et al., 2004; Sterjovski et al., 2011). These regions included the C2 region, the CD4

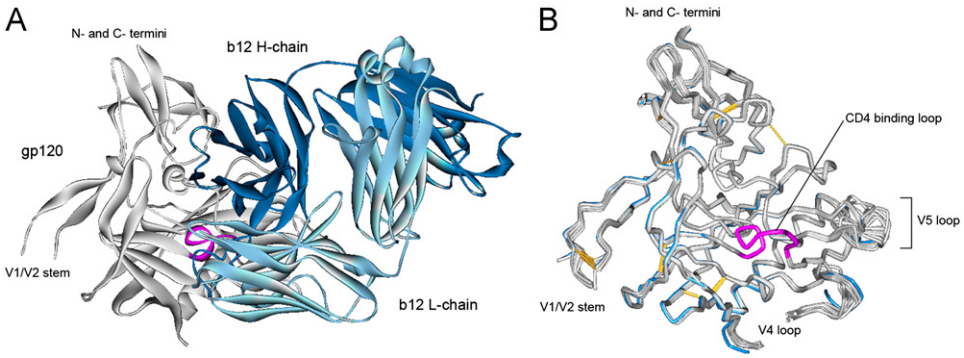

Fig. 1. Primary gp120 proteins in complex with b12 Fab. Models of gp120 proteins from primary HIV-1 isolates were generated by homology modeling using a crystal structure of gp120 bound to a b12 Fab fragment as a template (Zhou et al., 2007) (A). The heavy and light chains of the b12 Fab are shown in dark blue and light blue, respectively. All of the 16 primary gp120 models were superimposed onto the crystal structure (B); model and crystal structures are shown in Cα stick representation with primary gp120 models in grey and the crystal structure in blue. The CD4 binding loop is colored magenta and disulfide bonds in orange. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
RMSD of gp120 models aligned against the 2NYC crystal structure template.

Primary gp120 model	Cα atom RMSD (Å) [a]	Binding (RMFI)[b]	
		b12	BB10
NB2-C1	0.22	+++	+++
NB2-C4	0.28	++	+++
NB6-C3	0.39	+++	+++
NB6-C4	0.44	+++	+++
NB7-C1	0.29	+++	+++
NB7-C2	0.26	+++	+++
NB8-C2	0.26	+++	+++
NB8-C4	0.31	+++	+++
NB23-C2	0.31	+++	+++
NB23-C3	0.29	++	+++
NB24-C3	0.32	–	+++
NB24-C4	0.33	–	+++
NB25-C2	0.24	+++	+++
NB25-C3	0.28	+++	+++
NB27-C2	0.35	+++	+++
NB27-C3	0.32	+++	+++

Binding was scored as:–(0–50 RMFI), + (>200 RMFI), ++ (>1000 RMFI), +++(>2000 RMFI).

[a] Cα atom RMSD of the entire gp120 model structure was calculated based on all residues for each model.

[b] gp120 binding to b12 and the polyclonal HIV+ sera BB10 was analyzed by flow cytometry and expressed as relative mean fluorescence intensity (RMFI), as described in Materials and Methods.

binding loop motif, the C4 region and the V5 loop. The b12 epitope also spanned regions of gp120 that were not previously identified to be involved in CD4 binding, including an additional residue in C2, residues at the start of V4, and additional residues in C4. Analysis of the gp120–b12 interface highlighted that while the b12 epitope encompasses several regions involved in CD4 binding, it also contains regions of gp120 that are distinct from the CD4 binding site (Bublil et al., 2006; Zhou et al., 2007), suggesting that the CD4 binding site and b12 epitope are differentially modulated by amino acid variations at the surface of the interface. Based on the results of previous studies that have characterized the influence of Env alterations on CD4 binding, b12 binding and fusogenicity (Duenas-Decamp et al., 2009; Dunfee et al., 2006, 2007; Sterjovski et al., 2007, 2011), 5 amino acid variants at the gp120–b12 interface were identified and selected for further study. These included N279D and T283N within the C2 region (Fig. 2D), N362K/Q and Q363P/H/S adjacent to the CD4 binding loop motif (SSGGDPEI) within C4 (Fig. 2E), and N386D in V4 (Fig. 2F).

Polymorphisms within – and adjacent to – the b12 epitope in gp120 associated with b12 binding

Amino acid variants located at the gp120–b12 interface were then mapped onto the 2NY7 crystal structure (Fig. 3A) to illustrate their proximity to the b12 epitope. To investigate the potential of these variants to modulate the efficiency of b12 binding to gp120, binding assays were conducted for each of the primary Envs, the results of which are summarized in Table 1, and the relative abilities of gp120 to bind b12 was stratified according to the presence or absence of each variant (Fig. 3B–F). These analyses revealed that two amino acid variants, N362 (Fig. 3D) and Q363 (Fig. 3E), were associated with statistically significant increases in b12 binding. Positive trends were observed for the presence of N279 (Fig. 3B), T283 (Fig. 3C) and loss of a glycan at position 386 (Fig. 3F), however these were not statistically significant. Given that the Env proteins present in these binding assays are likely to contain some uncleaved gp160 as well as monomeric gp120 (Gorry et al., 2002), and since previous studies have shown that b12 may recognize monomeric gp120 more efficiently than gp160 (Roben et al., 1994), further biochemical studies using purified gp120 may be required to better understand the significance of the association between these Env amino acid variants and b12 binding.

We have previously identified N362 as being associated with more efficient cell–cell fusion and enhanced CD4 binding to gp120 (Sterjovski et al., 2007, 2011). Glycosylation of N362 could have a significant affect on b12 binding due to its position relative to the b12 epitope, in particular the CD4 binding loop which is an important structural element required for the gp120–b12 interaction. However, the mechanism by which N362 is associated with augmented b12 binding is unclear given the absence of direct interactions with b12.

The gp120 variant Q363 is also located adjacent to the CD4 binding loop. The amide side chain of glutamine has weakly basic properties, which can act as a H-bond acceptor. However, analysis of potential interacting partners of Q363 and other amino acids occurring at this position did not identify any potential contacts with b12 (data not shown). Absence of an atomic level explanation for N362 or Q363 may indicate that conformation or flexibility of the CD4 binding loop is likely to be the key to b12 recognition.

The immune response against HIV-1 is continually evolving over the course of infection, which in turn forces HIV-1 to adapt in order to escape neutralization (Richman et al., 2003). A study that examined the emergence of escape virus in response to neutralizing antibodies reported that neutralization escape mutations

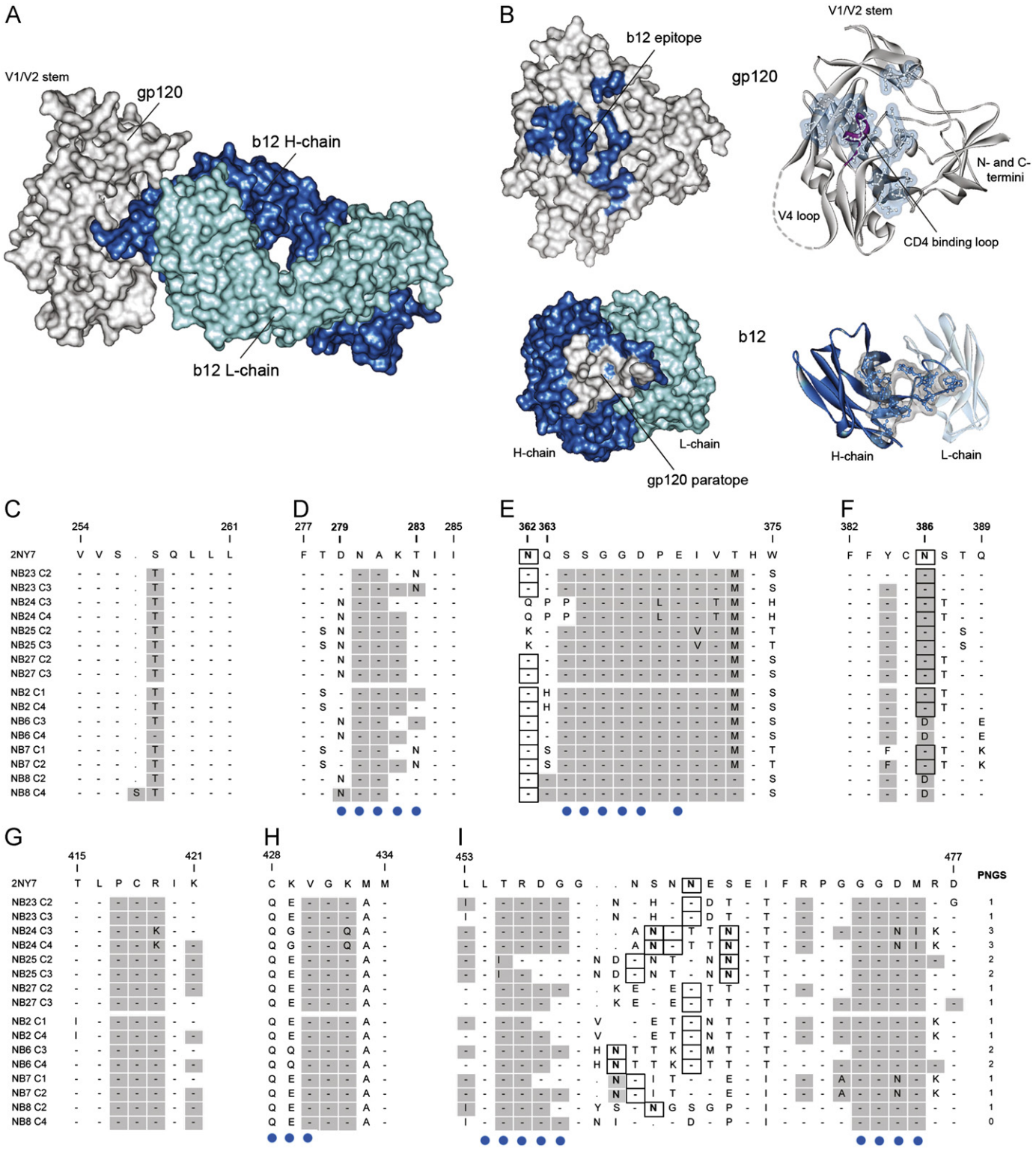

Fig. 2. Predicted b12 contact residues in gp120 structural models and identification of b12 epitope polymorphisms. The molecular surface of the 2NY7 crystal structure, with gp120 (white) shown bound to the heavy (dark blue) and light (light blue) chains of the b12 Fab fragment (A). The gp120–b12 interface was characterized using the PISA computational platform (Krissinel and Henrick, 2007) (B); the b12 epitope is shown in blue on the molecular surface (B, top left) and ribbon model (B, top right) of gp120 while the gp120 footprint on the heavy chain of the b12 Fab is shown in grey on the molecular surface (B, bottom left) and ribbon diagram (B, top right) of b12. In the ribbon diagrams, gp120 and the b12 heavy and light chains are colored as in (A), the CD4 binding loop is colored magenta and residues located within the gp120/b12 interface are shown as ball and stick models. Sequences extracted from primary gp120 models were aligned against the 2NY7 crystal structure sequence (C-I). The alignments illustrate gp120 residues at the gp120–b12 interface (shaded in grey) located in the C2 (C and D), C3 (E), V4 (F), C4 (G and H) and V5 (I) regions. Boxed residues indicate the asparagine residues that are within a potential N-linked glycosylated site (PNGS). Blue circles denote residues located within the CD4 binding site (Sterjovski et al., 2011). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

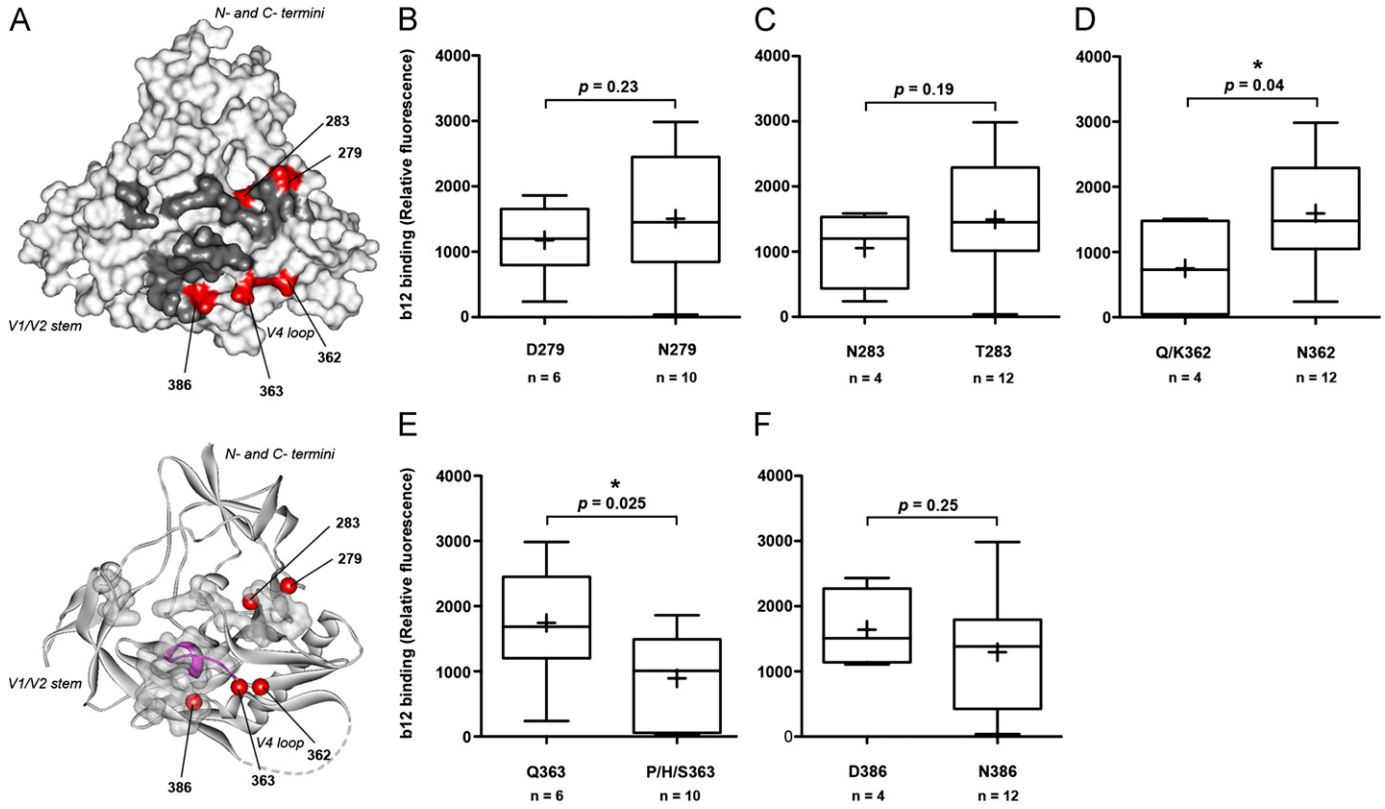

Fig. 3. b12 binding studies and amino acid variants located at the gp120–b12 interface. The b12 epitope (grey) and position of amino acid variants (red) displayed on the molecular surface of the 2NY7 crystal structure of gp120 (white) (A, top). The b12 epitope is also shown as grey molecular surface superimposed onto the ribbon model of gp120 (A, bottom) with Cα atoms of amino acid variants at position 278, 283, 362, 363 and 386 shown as red space-filling models and the CD4 binding loop colored magenta. b12 binding was determined by flow cytometry and values were stratified according to the absence or presence of amino acid polymorphisms at position 278 (B), 283 (C), 362 (D), 363 (E) and 386 (F) using Prism 5.0 a (Graphpad Software, San Diego CA). Box plots represent upper and lower quartiles and median scores, and whiskers represent minimum and maximum values and were constructed from mean values of duplicate experiments with each Env. The data shown are representative of 2 independent experiments. P values were calculated using an unpaired T test; values less than 0.05 were considered statistically significant. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

occurred predominantly in N-linked glycosylation sites, giving rise to the "evolving glycan shield" model of HIV-1 escape from immune selection pressures (Wei et al., 2003). Changes in the glycosylation pattern led to changes in glycan packing that allowed neutralizing antibody escape without inhibiting interactions with entry receptors. Therefore, shifts in glycosylation pattern have the potential to modulate b12 antibody binding. The N386D gp120 variant is associated with HIV-1 dementia and results in loss of a potential glycosylation site that was associated with reduced CD4 dependence and greater exposure of the CD4 binding site (Dunfee et al., 2007). While D386 had a minor effect on reduced CD4 affinity in this study, restoring the glycosylation site at this position resulted in a 8-fold increase in resistance to neutralisation by b12. More recently, glycosylation at position 386 was shown to be involved in immune evasion (Sanders et al., 2008). Interestingly, we show here that the V4 region, which contains the N386D variant, is involved in the formation of the gp120–b12 interface (Fig. 2F) but not the CD4 interface (Sterjovski et al., 2011). This may explain why D386 affected CD4 dependence to a small degree compared to the dramatic affect it had on sensitivity to neutralization by b12 (Dunfee et al., 2007).

Amino acid variants T283N and N279D have previously been associated with enhanced gp120–CD4 interactions. N283 is associated with enhanced CD4 affinity and macrophage tropism by brain-derived Envs (Duenas-Decamp et al., 2009; Dunfee et al., 2006) via the formation of an additional hydrogen bond between gp120 and Q40 of CD4 (Dunfee et al., 2006). However, mutagenesis studies suggest that its affect on b12 sensitivity may be context dependent (Duenas-Decamp et al., 2008). We have previously shown that the N279D mutation is associated with enhanced CD4 interaction and macrophage tropism that is most likely due to the introduction of a salt-bridge with K31 of CD4 (Sterjovski et al., 2011). In both cases, the variant associated with less efficient gp120–b12 binding has previously been linked to more efficient gp120–CD4 binding, further illustrating the opposing forces on CD4 and b12 binding in this region of Env.

Characterization of b12-resistant gp120 proteins

Two Env clones from NB24 demonstrated very low binding to b12 despite robust expression of Env on the surface of 293 T cells (Table 2). There is no significant difference between NB24 and NB8 Envs in binding to human recombinant soluble CD4 (Sterjovski et al., 2011), suggesting that differences between these Envs are restricted to the part of the b12 epitope that does not overlap the CD4 binding domain. Since b12 binding could affect sensitivity to neutralization of HIV-1 by b12, we next performed virus neutralization assays using luciferase reporter viruses pseudotyped with Envs that demonstrate very low (NB24-C3, NB24-C4) or very high (NB8-C2, NB8-C4) binding to b12, and the control R5 strains ADA and JR-CSF. An alternative potential outcome, however, is that the binding properties of monomeric gp120 to b12 may not necessarily predict the sensitivity of trimeric Env complexes that are present on the surface of virions to antibody neutralization sensitivity (Moore et al., 1995; Pantophlet et al., 2003; Parren et al., 1998; Roben et al., 1994; Sattentau and Moore,

1995). For example, conformational differences in the gp120 loop regions when they are part of the trimeric Env complex may affect the accessibility of b12 to its gp120 binding site, even though those regions are not part of the b12 epitope. The neutralization curves are shown in Fig. 4, and the IC_{50} and IC_{90} values are summarized alongside the b12 binding data (Table 2). Viruses pseudotyped with Envs from NB24 were resistant to neutralization by b12 (Fig. 4A), with neutralization curves failing to reach 50% inhibition at the highest concentration of b12. However, viruses pseudotyped with Envs from NB8 and the control R5 strains reached 100% neutralization by b12, with IC_{50} and IC_{90} values ranging between 0.9–2.9 and 3.8–5.5 μg of b12/ml, respectively (Table 2). In contrast, all the Env pseudotypes were readily neutralized by HIV-Ig (Fig. 4B).

Together, our results suggest that resistance to neutralization by b12 of NB24 Env clones is specific to the part of the b12 epitope that does not overlap the CD4 binding site. Our findings are consistent with those of previous studies of gp120 from different HIV-1 subtypes, which found that substitutions within the CD4 binding loop of gp120 as well as substitutions proximal to- but not part of the CD4 binding loop, can mediate resistance to neutralization by b12 (Wu et al., 2009). The latter substitutions permitted b12-resistant viruses to maintain strong CD4 binding capacity, similar to the NB8 and NB24 Envs studied here which differed in their b12 binding and neutralization sensitivities (Table 2), but maintained approximately equivalent levels of CD4 binding capacity (Sterjovski et al., 2011). Resistance of the NB24 Env clones to neutralization by b12 therefore provides a rare opportunity to probe the mechanisms of b12 resistance in isolation.

Table 2
Comparison of b12 binding and neutralization.

	Binding (RMFI)		Neutralization			
			b12 (μg/ml)		HIV-Ig (mg/ml)	
R5 Env	b12	BB10	IC_{50}	IC_{90}	IC_{50}	IC_{90}
Mock	–	–	n/a	n/a	n/a	n/a
ADA	+++	+++	1.3	3.9	1.4	2.2
JRCSF	+++	+++	0.9	3.8	0.6	1.3
NB24-C3	–	+++	>50	>50	1.0	2.4
NB24-C4	–	+++	>50	>50	0.8	2.0
NB8-C2	+++	+++	1.1	3.85	0.6	1.7
NB8-C4	+++	+++	1.3	3.92	0.3	1.2

gp120 binding to b12 and BB10 was analyzed by flow cytometry and expressed as relative mean fluorescence intensity (RMFI), as described in Materials and Methods. Binding was scored as:–(0–50 RMFI), + (>200 RMFI), ++ (>1000 RMFI), +++ (>2000 RMFI). Neutralization sensitivities to b12 and HIV-Ig were determined as described in Materials and Methods.

Structural features of gp120 associated with b12 resistance

To determine the structural features of gp120 associated with resistance or sensitivity to neutralization by b12, we next produced homology models of the b12-resistant clone NB24-C3 (Fig. 5A) and the b12-sensitive clone NB8-C2 (Fig. 5B) to map the b12 epitope in the context of the CD4 binding site. Residues located at the gp120–b12 interface (see Fig. 2) or those located within the CD4 binding site that were identified in our previous studies (Sterjovski et al., 2011), were shown as space-filling models on the gp120 ribbon structure and colored light blue or dark blue, respectively. Residues that overlap both the b12 and CD4 binding sites were colored magenta. Sequences extracted from the NB24-C3 and NB8-C2 model structures were aligned against the 2NY7 crystal structure sequence and colored as above (Fig. 5C). Since polymorphisms that involve the loss or shift of potential N-linked glycosylation sites (PNGS) are often associated with sensitivity to b12 neutralization, PNGS that differed between NB24-C3 and NB8-C2 were examined on the model structures (white space-filling models, Fig. 5A and B, right panels). One striking difference was the presence of the N386 glycan in the V4 loop of NB24-C3, which also mapped to the b12 epitope. While NB8-C2 contained PNGS in V4, they were not part of the b12 epitope. NB24-C3 also contained an insertion of a PNGS at position 460 located at the start of the V5 loop that was not present in NB8-C2. While N460 may contribute to exposure of residues in the CD4 and b12 binding sites, the location of N386 exclusively within the b12 epitope may contribute to the specific resistance to neutralization by b12 displayed by NB24 Env clones.

Amino acid alterations in the gp120 CD4 binding loop associated with b12 resistance

Other significant mutations in NB24 Env clones included amino acid changes located within or adjacent to the CD4 binding loop (Fig. 6). Close up views of the NB24-C3 and NB8-C2 CD4

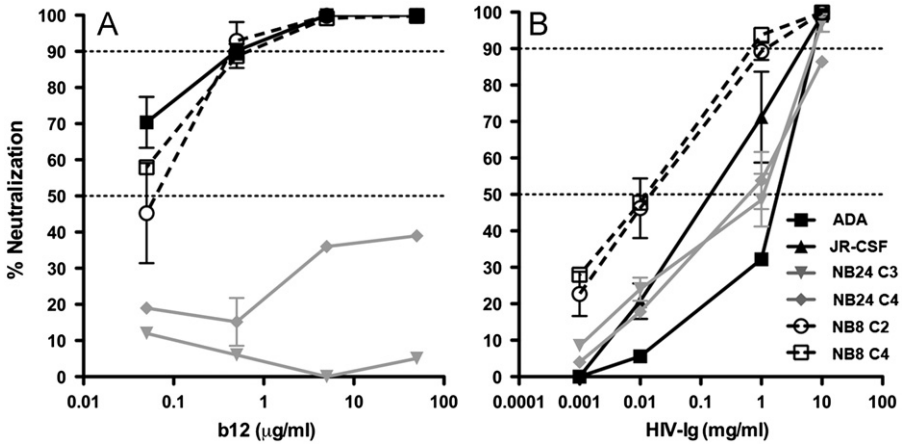

Fig. 4. Neutralization sensitivity of R5 Envs with low or high b12 binding. Luciferase reporter viruses pseudotyped with primary gp120 proteins exhibiting low b12 binding (NB24-C3 and NB24-C4) or high b12 binding (NB8-C2 and NB8-C4) were produced and quantified as described in Materials and Methods. Neutralization assays were performed using b12 (A) or HIV-Ig (B) and data were fitted with a non-linear function using Prism 5.0 a (Graphpad Software, San Diego CA). The data shown are means of triplicates, and the error bars represent standard deviations. The results shown are representative of 3 independent experiments.

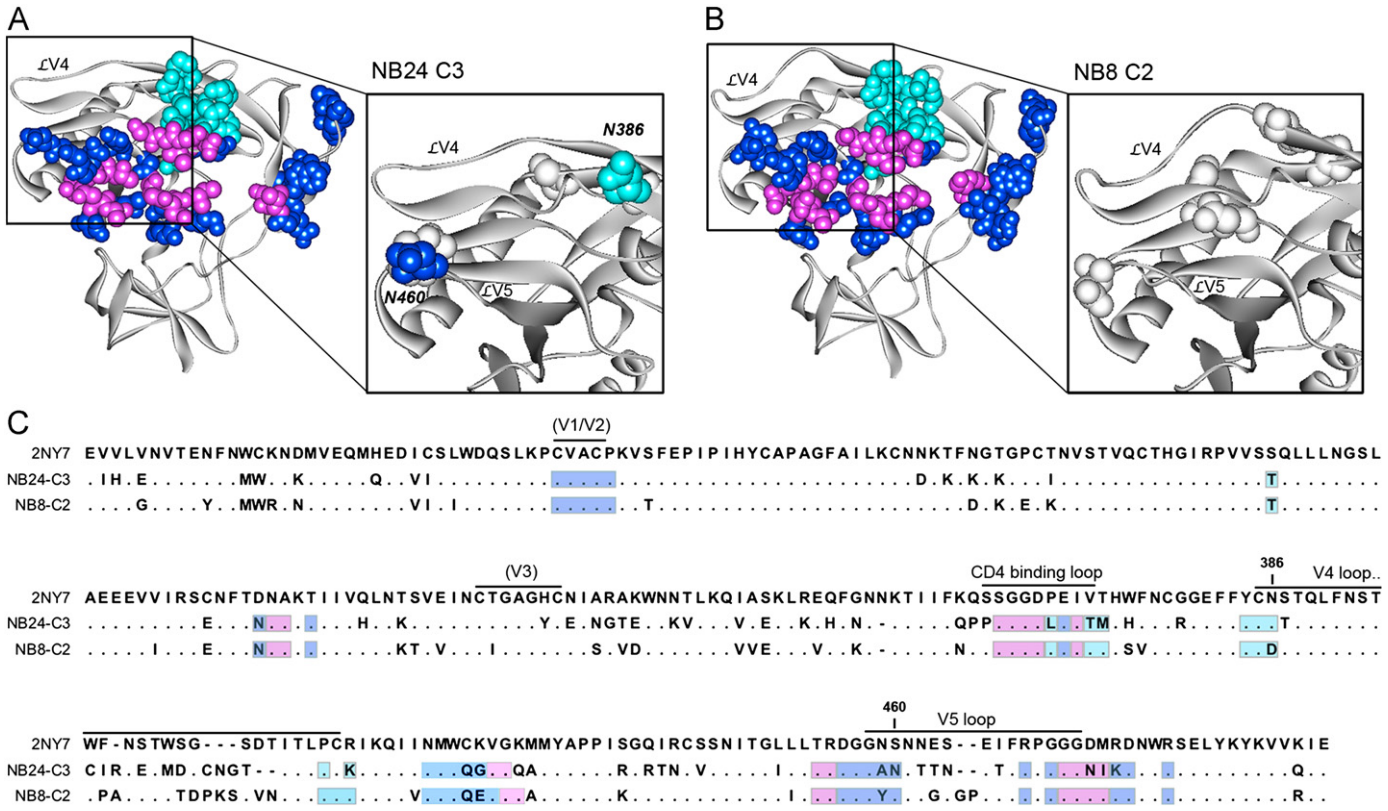

Fig. 5. Comparison of the b12-resistant NB24-C3 Env structure to the highly sensitive NB8-C2 Env structure in the context of the CD4 binding site and PNGS. Residues on NB24-C3 (A) and NB8-C2 (B) gp120 models (grey ribbons) located at the b12 and CD4 interface are shown as space-filling models and colored according to whether they are found exclusively within the b12 epitope (light blue), the CD4 binding site (dark blue) or whether they overlap both (magenta). Interface residues were determined as those located within 4Å of the ligand. Potential N-linked glycosylation sites colored white. (C) Multiple sequence alignment of sequences extracted from the NB24-C3 and NB8-C2 model structures were aligned against the 2NY7 crystal structure sequence and residues were colored as in panels A and B. Loop regions deleted from the crystal and model structures are labeled in parentheses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

binding loops are shown in Fig. 6B, with amino acid differences between the two Envs shown in stick representations and colored according to polarity. Amino acids P363, P364 and M373 in the *N*-terminal region of the NB24-C3 CD4 binding loop result in a more structurally rigid and nonpolar loop structure (Fig. 6B, top left) compared to the CD4 binding loop of NB8-C2 (Fig. 6B, right), which contains amino acids with flexible and polar side chains. The inherent conformational flexibility of gp120 is critical for antibody recognition (Kwong et al., 2002; Zhou et al., 2007). Substitutions of highly conserved residues in the CD4 binding loop with conformationally rigid amino acids such as proline may therefore contribute to the neutralization resistant phenotype of NB24 Env clones. In addition, analysis of potential interacting partners showed that the S364P change in NB24-C3 resulted in the loss of a hydrogen bond between gp120 and Y98 of b12 (Fig. 6B, lower left), which may contribute to the low affinity binding of b12 for NB24 clones. A serine at this position is highly conserved amongst the gp120 clones studied, with NB24 clones being the only sequences containing a proline at this position. Disruptions in hydrogen bond patterns in addition to the presence of inflexible and nonpolar residues in the CD4 binding loop of NB24 clones may restrict interactions with b12 and contribute to the resistant phenotype.

Comparisons between the CD4 binding loop of the highly neutralization resistant Env clones from NB24 and b12 sensitive clones from NB8 revealed that the serine to proline mutation at position 364 in NB24 Envs resulted in the loss of a hydrogen bond with Y98 in b12. Reduced flexibility as a result of the P363–P364 sequence in NB24 gp120 proteins may also affect binding of b12 to the CD4 binding loop. Both CD4 and b12 bind to the CD4 binding loop motif (Zhou et al., 2007). However, b12 uses all three complementarity determining region (CDR) heavy chain loops to tightly grasp the entire loop. It is therefore possible that mutations that limit the flexibility of the CD4 binding loop could significantly affect binding of the three b12 heavy chain loops. However, other structural features could also contribute to the highly b12 resistant phenotype of NB24 gp120 proteins. These features may be hidden in variable loops deleted from the original crystal structure on which the models were based (Zhou et al., 2007). The V1, V2 and V3 variable loops of gp120 are thought to be in close proximity to the CD4 binding site and movements of these loops have been associated with exposure of the coreceptor binding site upon CD4 binding (Chang et al., 2005; Dey et al., 2007; Steffens and Hope, 2004; Wyatt et al., 1998). Results from previous studies (Zwick et al., 2003) suggest that the V1, V2 and V3 loops, as well as C4 residues that form part of the bridging sheet, are in close enough proximity to one another and to the CD4 binding site to be involved in a single discontinuous epitope for the neutralizing antibody 4KG5. In addition, alterations of the V1, V2 and V3 loops have been previously shown to differentially modulate the response to antibodies directed against the CD4 binding site (Ly and Stamatatos, 2000; Nabatov et al., 2004). This highlights the possibility that gp120 variable loops excluded from the models studied here and from the available crystal structure (Zhou et al., 2007) may have significant roles in the ability of primary gp120 proteins to bind CD4 and b12, and may

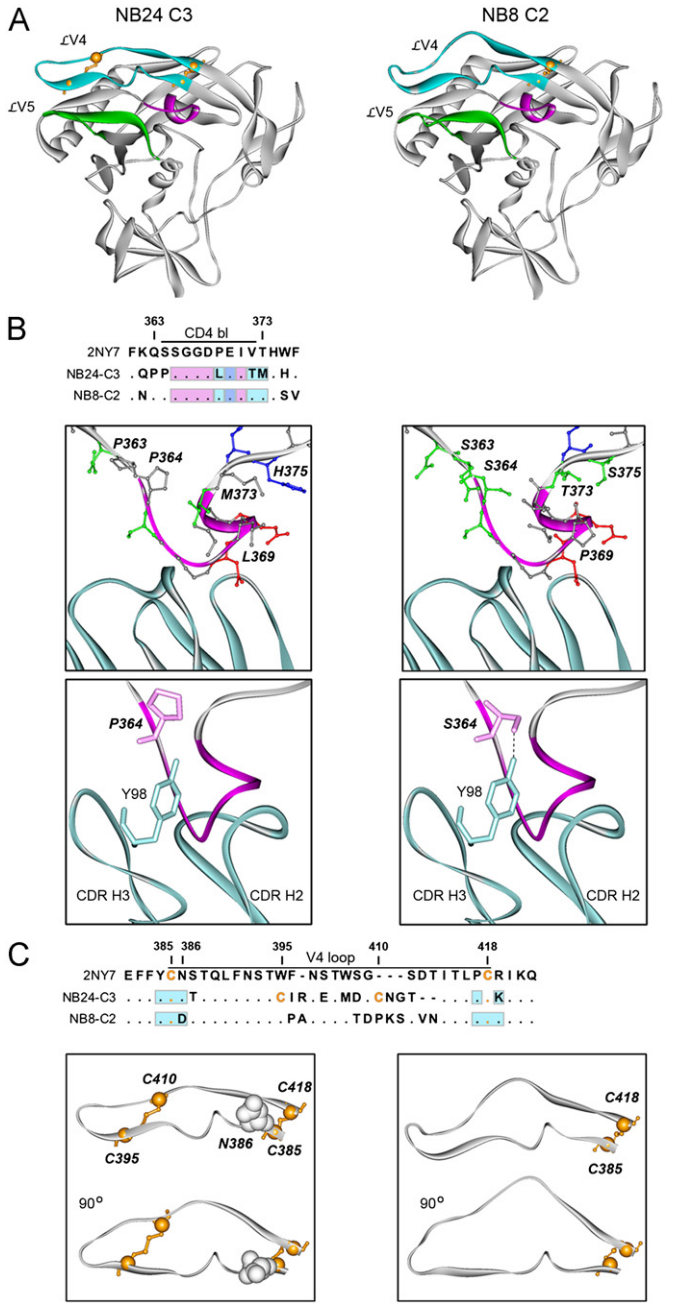

Fig. 6. Structural features of gp120 associated with resistance to neutralization by b12. gp120 protein structures (A, grey ribbon) of the b12 resistant clone NB24-C3 (left) and b12-sensitive clone NB8-C2 (right panels) showing the V4 loop (blue), V5 loop (green) and CD4 binding loop (magenta). The Cα atoms of cysteine residues involved in disulfide bonds in the V4 loop are shown as orange space-filling models and the disulfide bond itself is shown in orange stick representation. Sequence alignment showing amino acid differences in the CD4 binding loop region of gp120 (B), also illustrated on the NB24-C3 (B, top left) and NB8-C2 (B, top right) protein models coloured as in (A), with the b12 ribbon shown in blue (CDR H2 loop hidden). Close up view of the CD4 binding loop showing the position of P364 in NB24-C3 clone (B, bottom left) and the hydrogen bond between S364 and Y98 on the b12 CDR H3 loop in NB8-C2 (B, bottom right). Sequence alignment of the V4 loop (C) showing the location of additional cysteine residues in NB24-C3 that form an additional disulfide bond in the model structure (C, left), compared to the single disulfide bond in NB8-C3 (C, right). N386 is shown as a white space-filling model and disulfide bonds modeled as described in panel A. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

contain structural features that could also contribute to the differences seen in the efficiency of CD4 use and sensitivity to neutralization by b12.

An additional disulfide bond in the gp120 V4 loop is associated with b12 resistance

The b12 resistant Env clones from NB24 also contain an additional disulfide bond in the gp120 V4 loop via the introduction of two cysteine residues at positions 395 and 410 (Fig. 6C, left panel), in addition to the highly conserved cysteine residues at position 385 and 418 at the base of the loop. Analysis of 177 gp120 sequences published in the 2009 Los Alamos HIV Sequence Compendium revealed that the presence of an additional disulfide bond in the V4 loop occurred in only 9% of sequences (14 out of 177), suggesting that this is a relatively uncommon structural feature in HIV-1 gp120 proteins. An additional disulfide bond in the V4 loop, which contains residues that are involved in formation of the b12 epitope, including the immunomodulatory glycan N386, is likely to affect the conformational flexibiliy of the b12 recognition site.

Conclusions

In summary, the three-dimensional models of b12-bound primary R5 gp120 proteins studied here provided valuable insights into the structural determinants of an important HIV-1 neutralization epitope. By mapping the b12 structural epitope to the amino acid sequences of primary gp120 proteins with a range of b12-binding and b12-neutralization sensitivities, we identified polymorphisms at the gp120–b12 interface associated with sensitivity and resistance to b12. By comparing the b12 epitope to the overlapping yet distinct CD4 binding site we were able to identify regions that were important for b12 resistance that mapped exclusively to the b12 epitope. Furthermore, our results highlight the importance of gp120 structural flexibility for efficient presentation of the b12 epitope. Together, our results provide new mechanistic insights into the interaction between physiologically-relevant R5 gp120 proteins and b12, which may be significant for structure-guided approaches to HIV-1 vaccine design that depend on a thorough knowledge of the presentation of neutralization epitopes in gp120.

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References

Bublil, E.M., Yeger-Azuz, S., Gershoni, J.M., 2006. Computational prediction of the cross-reactive neutralizing epitope corresponding to the [corrected] monclonal [corrected] antibody b12 specific for HIV-1 gp120. Faseb J. 20, 1762–1774.
Burton, D.R., Barbas 3rd, C.F., Persson, M.A., Koenig, S., Chanock, R.M., Lerner, R.A., 1991. A large array of human monoclonal antibodies to type 1 human

immunodeficiency virus from combinatorial libraries of asymptomatic seropositive individuals. Proc. Nat. Acad. Sci. U.S.A. 88, 10134–10137.
Burton, D.R., Pyati, J., Koduri, R., Sharp, S.J., Thornton, G.B., Parren, P.W., Sawyer, L.S., Hendry, R.M., Dunlop, N., Nara, P.L., et al., 1994. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science 266, 1024–1027.
Cashin, K., Roche, M., Sterjovski, J., Ellett, A., Gray, L.R., Cunningham, A.L., Ramsland, P.A., Churchill, M.J., Gorry, P.R., 2011. Alternative coreceptor requirements for efficient CCR5- and CXCR4-mediated HIV-1 entry into macrophages. J. Virol. 85, 10699–10709.
Chan, D.C., Fass, D., Berger, J.M., Kim, P.S., 1997. Core structure of gp41 from the HIV envelope glycoprotein. Cell 89, 263–273.
Chang, M.I., Panorchan, P., Dobrowsky, T.M., Tseng, Y., Wirtz, D., 2005. Single-molecule analysis of human immunodeficiency virus type 1 gp120-receptor interactions in living cells. J. Virol. 79, 14748–14755.
Dalgleish, A.G., Beverley, P.C., Clapham, P.R., Crawford, D.H., Greaves, M.F., Weiss, R.A., 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 312, 763–767.
Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R.E., Hill, C.M., Davis, C.B., Peiper, S.C., Schall, T.J., Littman, D.R., Landau, N.R., 1996. Identification of a major co-receptor for primary isolates of HIV-1. Nature 381, 661–666.
Dey, B., Pancera, M., Svehla, K., Shu, Y., Xiang, S.H., Vainshtein, J., Li, Y., Sodroski, J., Kwong, P.D., Mascola, J.R., Wyatt, R., 2007. Characterization of human immunodeficiency virus type 1 monomeric and trimeric gp120 glycoproteins stabilized in the CD4-bound state: antigenicity, biophysics, and immunogenicity. J. Virol. 81, 5579–5593.
Dragic, T., Litwin, V., Allaway, G.P., Martin, S.R., Huang, Y., Nagashima, K.A., Cayanan, C., Maddon, P.J., Koup, R.A., Moore, J.P., Paxton, W.A., 1996. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature 381, 667–673.
Duenas-Decamp, M.J., Peters, P., Burton, D., Clapham, P.R., 2008. Natural resistance of human immunodeficiency virus type 1 to the CD4bs antibody b12 conferred by a glycan and an arginine residue close to the CD4 binding loop. J. Virol. 82, 5807–5814.
Duenas-Decamp, M.J., Peters, P.J., Burton, D., Clapham, P.R., 2009. Determinants flanking the CD4 binding loop modulate macrophage tropism of human immunodeficiency virus type 1 R5 envelopes. J. Virol. 83, 2575–2583.
Dunfee, R.L., Thomas, E.R., Gorry, P.R., Wang, J., Taylor, J., Kunstman, K., Wolinsky, S.M., Gabuzda, D., 2006. The HIV Env variant N283 enhances macrophage tropism and is associated with brain infection and dementia. Proc. Nat. Acad. Sci. U.S.A. 103, 15160–15165.
Dunfee, R.L., Thomas, E.R., Wang, J., Kunstman, K., Wolinsky, S.M., Gabuzda, D., 2007. Loss of the N-linked glycosylation site at position 386 in the HIV envelope V4 region enhances macrophage tropism and is associated with dementia. Virology 367, 222–234.
Etemad-Moghadam, B., Sun, Y., Nicholson, E.K., Fernandes, M., Liou, K., Gomila, R., Lee, J., Sodroski, J., 2000. Envelope glycoprotein determinants of increased fusogenicity in a pathogenic simian-human immunodeficiency virus (SHIV-KB9) passaged in vivo. J. Virol. 74, 4433–4440.
Feng, Y., Broder, C.C., Kennedy, P.E., Berger, E.A., 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 272, 872–877.
Gao, F., Morrison, S.G., Robertson, D.L., Thornton, C.L., Craig, S., Karlsson, G., Sodroski, J., Morgado, M., Galvao-Castro, B., von Briesen, H., Beddows, S., Weber, J., Sharp, P.M., Shaw, G.M., Hahn, B.H., 1996. Molecular cloning and analysis of functional envelope genes from human immunodeficiency virus type 1 sequence subtypes A through G. The WHO and NIAID Networks for HIV Isolation and Characterization. J. Virol. 70, 1651–1667.
Gorry, P.R., Dunfee, R.L., Mefford, M.E., Kunstman, K., Morgan, T., Moore, J.P., Mascola, J.R., Agopian, K., Holm, G.H., Mehle, A., Taylor, J., Farzan, M., Wang, H., Ellery, P., Willey, S.J., Clapham, P.R., Wolinsky, S.M., Crowe, S.M., Gabuzda, D., 2007. Changes in the V3 region of gp120 contribute to unusually broad coreceptor usage of an HIV-1 isolate from a CCR5 Delta32 heterozygote. Virology 362, 163–178.
Gorry, P.R., Howard, J.L., Churchill, M.J., Anderson, J.L., Cunningham, A., Adrian, D., McPhee, D.A., Purcell, D.F., 1999. Diminished production of human immunodeficiency virus type 1 in astrocytes results from inefficient translation of gag, env, and nef mRNAs despite efficient expression of Tat and Rev. J. Virol. 73, 352–361.
Gorry, P.R., Taylor, J., Holm, G.H., Mehle, A., Morgan, T., Cayabyab, M., Farzan, M., Wang, H., Bell, J.E., Kunstman, K., Moore, J.P., Wolinsky, S.M., Gabuzda, D., 2002. Increased CCR5 affinity and reduced CCR5/CD4 dependence of a neurovirulent primary human immunodeficiency virus type 1 isolate. J. Virol. 76, 6277–6292.
Gray, L., Churchill, M.J., Keane, N., Sterjovski, J., Ellett, A.M., Purcell, D.F., Poumbourios, P., Kol, C., Wang, B., Saksena, N.K., Wesselingh, S.L., Price, P., French, M., Gabuzda, D., Gorry, P.R., 2006. Genetic and functional analysis of R5 × 4 human immunodeficiency virus type 1 envelope glycoproteins derived from two individuals homozygous for the CCR5delta32 allele. J. Virol. 80, 3684–3691.
Gray, L., Roche, M., Churchill, M.J., Sterjovski, J., Ellett, A., Poumbourios, P., Sheffief, S., Wang, B., Saksena, N., Purcell, D.F., Wesselingh, S., Cunningham, A.L., Brew, B.J., Gabuzda, D., Gorry, P.R., 2009. Tissue-specific sequence alterations in the human immunodeficiency virus type 1 envelope favoring CCR5 usage contribute to persistence of dual-tropic virus in the brain. J. Virol. 83, 5430–5441.
Gray, L., Sterjovski, J., Churchill, M., Ellery, P., Nasr, N., Lewin, S.R., Crowe, S.M., Wesselingh, S.L., Cunningham, A.L., Gorry, P.R., 2005. Uncoupling coreceptor usage of human immunodeficiency virus type 1 (HIV-1) from macrophage tropism reveals biological properties of CCR5-restricted HIV-1 isolates from patients with acquired immunodeficiency syndrome. Virology 337, 384–398.
Kozarsky, K., Penman, M., Basiripour, L., Haseltine, W., Sodroski, J., Krieger, M., 1989. Glycosylation and processing of the human immunodeficiency virus type 1 envelope protein. J. Acquir. Immune. Defic. Syndr. 2, 163–169.
Krissinel, E., Henrick, K., 2007. Inference of macromolecular assemblies from crystalline state. J. Mol. Biol. 372, 774–797.
Kwong, P.D., Doyle, M.L., Casper, D.J., Cicala, C., Leavitt, S.A., Majeed, S., Steenbeke, T.D., Venturi, M., Chaiken, I., Fung, M., Katinger, H., Parren, P.W., Robinson, J., Van Ryk, D., Wang, L., Burton, D.R., Freire, E., Wyatt, R., Sodroski, J., Hendrickson, W.A., Arthos, J., 2002. HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature 420, 678–682.
Kwong, P.D., Wyatt, R., Majeed, S., Robinson, J., Sweet, R.W., Sodroski, J., Hendrickson, W.A., 2000a. Structures of HIV-1 gp120 envelope glycoproteins from laboratory-adapted and primary isolates. Struct. Fold. Des. 8, 1329–1339.
Kwong, P.D., Wyatt, R., Robinson, J., Sweet, R.W., Sodroski, J., Hendrickson, W.A., 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 393, 648–659.
Kwong, P.D., Wyatt, R., Sattentau, Q.J., Sodroski, J., Hendrickson, W.A., 2000b. Oligomeric modeling and electrostatic analysis of the gp120 envelope glycoprotein of human immunodeficiency virus. J. Virol. 74, 1961–1972.
Li, Y., O'Dell, S., Walker, L.M., Wu, X., Guenaga, J., Feng, Y., Schmidt, S.D., McKee, K., Louder, M.K., Ledgerwood, J.E., Graham, B.S., Haynes, B.F., Burton, D.R., Wyatt, R.T., Mascola, J.R., 2011. Mechanism of neutralization by the broadly neutralizing HIV-1 monoclonal antibody VRC01. J. Virol. 85, 8954–8967.
Liu, J., Bartesaghi, A., Borgnia, M.J., Sapiro, G., Subramaniam, S., 2008. Molecular architecture of native HIV-1 gp120 trimers. Nature 455, 109–113.
Ly, A., Stamatatos, L., 2000. V2 loop glycosylation of the human immunodeficiency virus type 1 SF162 envelope facilitates interaction of this protein with CD4 and CCR5 receptors and protects the virus from neutralization by anti-V3 loop and anti-CD4 binding site antibodies. J. Virol. 74, 6769–6776.
McCaffrey, R.A., Saunders, C., Hensel, M., Stamatatos, L., 2004. N-linked glycosylation of the V3 loop and the immunologically silent face of gp120 protects human immunodeficiency virus type 1 SF162 from neutralization by anti-gp120 and anti-gp41 antibodies. J. Virol. 78, 3279–3295.
McInerney, T.L., McLain, L., Armstrong, S.J., Dimmock, N.J., 1997. A human IgG1 (b12) specific for the CD4 binding site of HIV-1 neutralizes by inhibiting the virus fusion entry process, but b12 Fab neutralizes by inhibiting a postfusion event. Virology 233, 313–326.
Moore, J.P., Cao, Y., Qing, L., Sattentau, Q.J., Pyati, J., Koduri, R., Robinson, J., Barbas 3rd, C.F., Burton, D.R., Ho, D.D., 1995. Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120. J. Virol. 69, 101–109.
Muster, T., Guinea, R., Trkola, A., Purtscher, M., Klima, A., Steindl, F., Palese, P., Katinger, H., 1994. Cross-neutralizing activity against divergent human immunodeficiency virus type 1 isolates induced by the gp41 sequence ELDKWAS. J. Virol. 68, 4031–4034.
Nabatov, A.A., Pollakis, G., Linnemann, T., Kliphius, A., Chalaby, M.I., Paxton, W.A., 2004. Intrapatient alterations in the human immunodeficiency virus type 1 gp120 V1V2 and V3 regions differentially modulate coreceptor usage, virus inhibition by CC/CXC chemokines, soluble CD4, and the b12 and 2G12 monoclonal antibodies. J. Virol. 78, 524–530.
Pantophlet, R., Ollmann Saphire, E., Poignard, P., Parren, P.W., Wilson, I.A., Burton, D.R., 2003. Fine mapping of the interaction of neutralizing and nonneutralizing monoclonal antibodies with the CD4 binding site of human immunodeficiency virus type 1 gp120. J. Virol. 77, 642–658.
Parren, P.W., Marx, P.A., Hessell, A.J., Luckay, A., Harouse, J., Cheng-Mayer, C., Moore, J.P., Burton, D.R., 2001. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. J. Virol. 75, 8340–8347.
Parren, P.W., Mondor, I., Naniche, D., Ditzel, H.J., Klasse, P.J., Burton, D.R., Sattentau, Q.J., 1998. Neutralization of human immunodeficiency virus type 1 by antibody to gp120 is determined primarily by occupancy of sites on the virion irrespective of epitope specificity. J. Virol. 72, 3512–3519.
Platt, E.J., Wehrly, K., Kuhmann, S.E., Chesebro, B., Kabat, D., 1998. Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1. J. Virol. 72, 2855–2864.
Richman, D.D., Wrin, T., Little, S.J., Petropoulos, C.J., 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc. Nat. Acad. Sci. U.S.A. 100, 4144–4149.
Rizzuto, C.D., Wyatt, R., Hernandez-Ramos, N., Sun, Y., Kwong, P.D., Hendrickson, W.A., Sodroski, J., 1998. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. Science 280, 1949–1953.
Roben, P., Moore, J.P., Thali, M., Sodroski, J., Barbas 3rd, C.F., Burton, D.R., 1994. Recognition properties of a panel of human recombinant Fab fragments to the CD4 binding site of gp120 that show differing abilities to neutralize human immunodeficiency virus type 1. J. Virol. 68, 4821–4828.
Roche, M., Jakobsen, M.R., Ellett, A., Salimiseyedabad, H., Jubb, B., Westby, M., Lee, B., Lewin, S.R., Churchill, M.J., Gorry, P.R., 2011a. HIV-1 predisposed to acquiring resistance to maraviroc (MVC) and other CCR5 antagonists in vitro has an inherent, low-level ability to utilize MVC-bound CCR5 for entry. Retrovirology 8, 89.

Roche, M., Jakobsen, M.R., Sterjovski, J., Ellett, A., Posta, F., Lee, B., Jubb, B., Westby, M., Lewin, S.R., Ramsland, P.A., Churchill, M.J., Gorry, P.R., 2011b. HIV-1 escape from the CCR5 antagonist maraviroc associated with an altered and less efficient mechanism of gp120–CCR5 engagement that attenuates macrophage-tropism. J. Virol. 85, 4330–4342.

Sali, A., Blundell, T.L., 1993. Comparative protein modelling by satisfaction of spatial restraints. J. Mol. Biol. 234, 779–815.

Sanders, R.W., van Anken, E., Nabatov, A.A., Liscaljet, I.M., Bontjer, I., Eggink, D., Melchers, M., Busser, E., Dankers, M.M., Groot, F., Braakman, I., Berkhout, B., Paxton, W.A., 2008. The carbohydrate at asparagine 386 on HIV-1 gp120 is not essential for protein folding and function but is involved in immune evasion. Retrovirology 5, 10.

Sattentau, Q., 1998. HIV gp120: double lock strategy foils host defences. Structure 6, 945–949.

Sattentau, Q.J., Moore, J.P., 1991. Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. J. Exp. Med. 174, 407–415.

Sattentau, Q.J., Moore, J.P., 1995. Human immunodeficiency virus type 1 neutralization is determined by epitope exposure on the gp120 oligomer. J. Exp. Med. 182, 185–196.

Steffens, C.M., Hope, T.J., 2004. Mobility of the human immunodeficiency virus (HIV) receptor CD4 and coreceptor CCR5 in living cells: implications for HIV fusion and entry events. J. Virol. 78, 9573–9578.

Sterjovski, J., Churchill, M.J., Ellett, A., Gray, L.R., Roche, M.J., Dunfee, R.L., Purcell, D.F., Saksena, N., Wang, B., Sonza, S., Wesselingh, S.L., Karlsson, I., Fenyo, E.M., Gabuzda, D., Cunningham, A.L., Gorry, P.R., 2007. Asn 362 in gp120 contributes to enhanced fusogenicity by CCR5-restricted HIV-1 envelope glycoprotein variants from patients with AIDS. Retrovirology 4, 89.

Sterjovski, J., Churchill, M.J., Roche, M., Ellett, A., Farrugia, W., Wesselingh, S.L., Cunningham, A.L., Ramsland, P.A., Gorry, P.R., 2011. CD4-binding site alterations in CCR5-using HIV-1 envelopes influencing gp120–CD4 interactions and fusogenicity. Virology 410, 418–428.

Sterjovski, J., Roche, M., Churchill, M.J., Ellett, A., Farrugia, W., Gray, L.R., Cowley, D., Poumbourios, P., Lee, B., Wesselingh, S., Cunningham, A.L., Ramsland, P.A., Gorry, P.R., 2010. An altered and more efficient mechanism of CCR5 engagement contributes to macrophage tropism of CCR5-using HIV-1 envelopes. Virology 404, 269–278.

Thali, M., Moore, J.P., Furman, C., Charles, M., Ho, D.D., Robinson, J., Sodroski, J., 1993. Characterization of conserved human immunodeficiency virus type 1 gp120 neutralization epitopes exposed upon gp120–CD4 binding. J. Virol. 67, 3978–3988.

Trkola, A., Dragic, T., Arthos, J., Binley, J.M., Olson, W.C., Allaway, G.P., Cheng-Mayer, C., Robinson, J., Maddon, P.J., Moore, J.P., 1996a. CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. Nature 384, 184–187.

Trkola, A., Pomales, A.B., Yuan, H., Korber, B., Maddon, P.J., Allaway, G.P., Katinger, H., Barbas 3rd, C.F., Burton, D.R., Ho, D.D., et al., 1995. Cross-clade neutralization of primary isolates of human immunodeficiency virus type 1 by human monoclonal antibodies and tetrameric CD4-IgG. J. Virol. 69, 6609–6617.

Trkola, A., Purtscher, M., Muster, T., Ballaun, C., Buchacher, A., Sullivan, N., Srinivasan, K., Sodroski, J., Moore, J.P., Katinger, H., 1996b. Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. J Virol. 70, 1100–1108.

Veazey, R.S., Shattock, R.J., Pope, M., Kirijan, J.C., Jones, J., Hu, Q., Ketas, T., Marx, P.A., Klasse, P.J., Burton, D.R., Moore, J.P., 2003. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. Nat. Med. 9, 343–346.

Wei, X., Decker, J.M., Wang, S., Hui, H., Kappes, J.C., Wu, X., Salazar-Gonzalez, J.F., Salazar, M.G., Kilby, J.M., Saag, M.S., Komarova, N.L., Nowak, M.A., Hahn, B.H., Kwong, P.D., Shaw, G.M., 2003. Antibody neutralization and escape by HIV-1. Nature 422, 307–312.

Wu, L., Gerard, N.P., Wyatt, R., Choe, H., Parolin, C., Ruffing, N., Borsetti, A., Cardoso, A.A., Desjardin, E., Newman, W., Gerard, C., Sodroski, J., 1996. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. Nature 384, 179–183.

Wu, X., Zhou, T., O'Dell, S., Wyatt, R.T., Kwong, P.D., Mascola, J.R., 2009. Mechanism of human immunodeficiency virus type 1 resistance to monoclonal antibody B12 that effectively targets the site of CD4 attachment. J. Virol. 83, 10892–10907.

Wyatt, R., Kwong, P.D., Desjardins, E., Sweet, R.W., Robinson, J., Hendrickson, W.A., Sodroski, J.G., 1998. The antigenic structure of the HIV gp120 envelope glycoprotein. Nature 393, 705–711.

Wyatt, R., Moore, J., Accola, M., Desjardin, E., Robinson, J., Sodroski, J., 1995. Involvement of the V1/V2 variable loop structure in the exposure of human immunodeficiency virus type 1 gp120 epitopes induced by receptor binding. J. Virol. 69, 5723–5733.

Wyatt, R., Sodroski, J., 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 280, 1884–1888.

Wyatt, R., Sullivan, N., Thali, M., Repke, H., Ho, D., Robinson, J., Posner, M., Sodroski, J., 1993. Functional and immunologic characterization of human immunodeficiency virus type 1 envelope glycoproteins containing deletions of the major variable regions. J. Virol. 67, 4557–4565.

Yang, X., Kurteva, S., Lee, S., Sodroski, J., 2005. Stoichiometry of antibody neutralization of human immunodeficiency virus type 1. J. Virol. 79, 3500–3508.

Yang, X., Tomov, V., Kurteva, S., Wang, L., Ren, X., Gorny, M.K., Zolla-Pazner, S., Sodroski, J., 2004. Characterization of the outer domain of the gp120 glycoprotein from human immunodeficiency virus type 1. J. Virol. 78, 12975–12986.

Yang, X., Wyatt, R., Sodroski, J., 2001. Improved elicitation of neutralizing antibodies against primary human immunodeficiency viruses by soluble stabilized envelope glycoprotein trimers. J. Virol. 75, 1165–1171.

Zhou, T., Xu, L., Dey, B., Hessell, A.J., Van Ryk, D., Xiang, S.H., Yang, X., Zhang, M.Y., Zwick, M.B., Arthos, J., Burton, D.R., Dimitrov, D.S., Sodroski, J., Wyatt, R., Nabel, G.J., Kwong, P.D., 2007. Structural definition of a conserved neutralization epitope on HIV-1 gp120. Nature 445, 732–737.

Zwick, M.B., Parren, P.W., Saphire, E.O., Church, S., Wang, M., Scott, J.K., Dawson, P.E., Wilson, I.A., Burton, D.R., 2003. Molecular features of the broadly neutralizing immunoglobulin G1 b12 required for recognition of human immunodeficiency virus type 1 gp120. J. Virol. 77, 5863–5876.